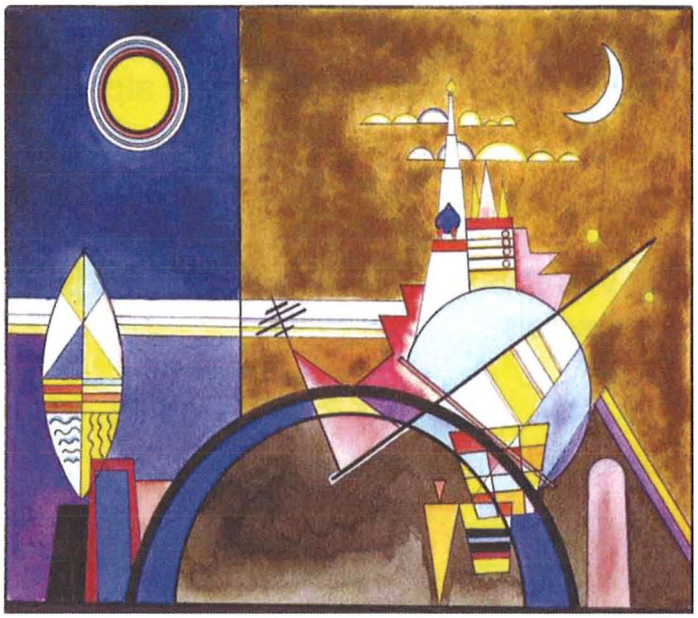
Alpha-1 Antitrypsin Deficiency:

Still Crazy After All These Years



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Internal Medicine Grand Rounds

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This is to acknowledge that Jonathan C. Weissler M.D. has disclosed financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Weissler will not be discussing off-label uses in his presentation.

Cover (left): The Great Gate of Kiev. Stage set for Mussorgsky's Pictures at an Exhibition.

Cover (right): Frederic Chopin (1810-49), thought to have died from alpha-1 antitrypsin deficiency[1]

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Interests: Pathogenesis of emphysema, pulmonary fibrosis, critical care medicine, conflict resolution.

Purpose and overview: Alpha-1 antitrypsin (AAT) deficiency is the result of mutations occurring in roughly 1 in 1500-5000 live births and is associated with clinical disease ranging from cirrhosis to emphysema to skin disease or vasculitis. The pathophysiology of disease differs amongst organ systems involved, with both loss of anti-protease function and a "toxic gain of function" from polymers of aberrant AAT playing a role. Proper therapy for AAT deficiency has important medical, lifestyle, and financial consequences and will be discussed.

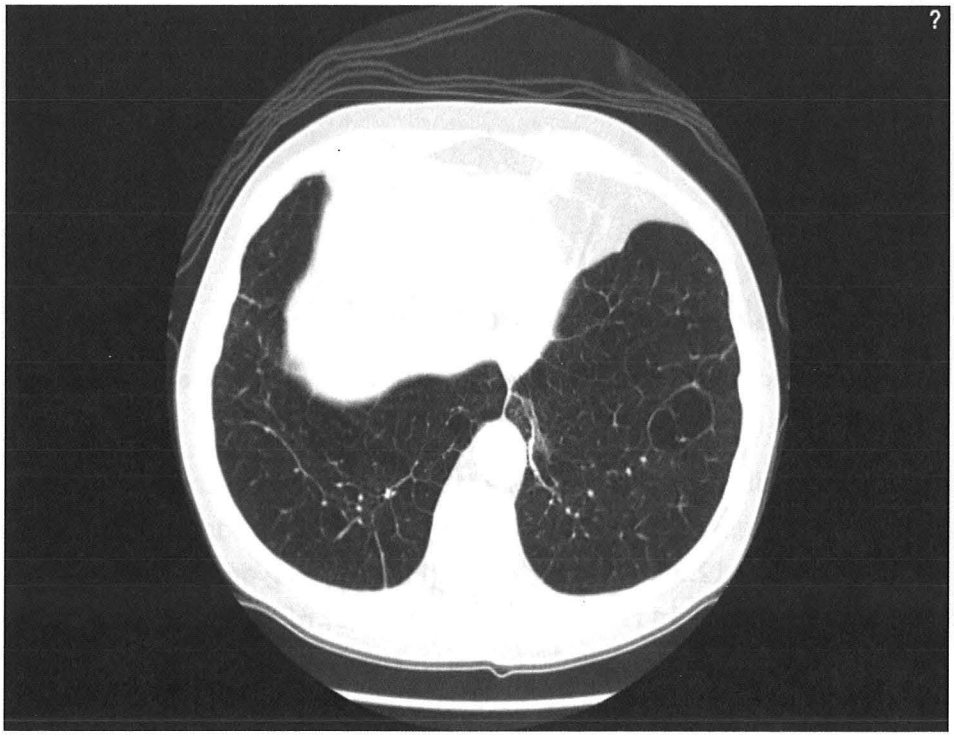
Objectives:

1. To review the incidence of alpha-1 antitrypsin (AAT) deficiency
2. To discuss the pathophysiology of lung and liver disease in AAT deficiency
3. To review the clinical spectrum of disease in AAT deficiency
4. To review the treatment options available for patients with AAT deficiency

Case Presentation:

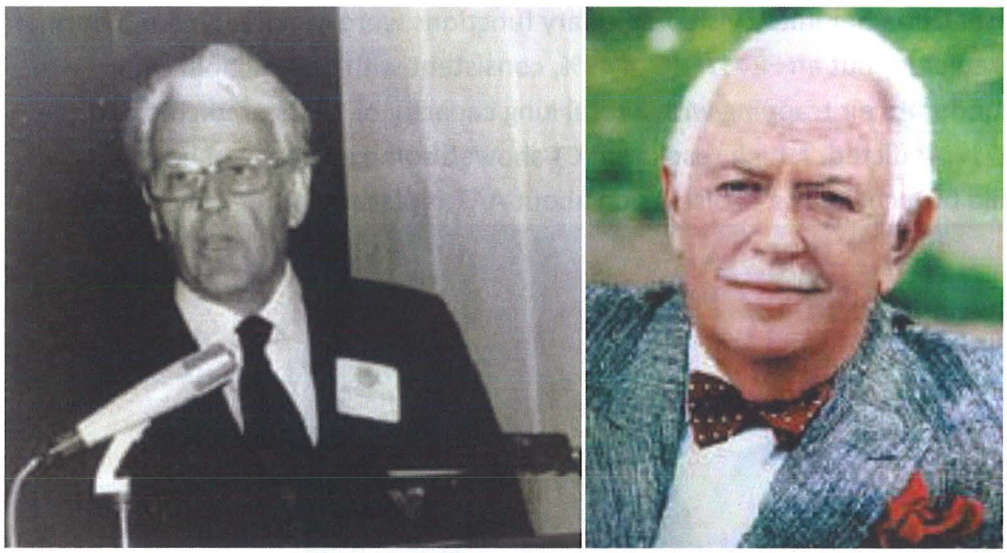
A.C. was a 58 year old woman with known ZZ phenotype of alpha-1 antitrypsin (AAT) deficiency when she was referred in February 2007 for a second opinion regarding AAT replacement therapy. Her mother had died of emphysema at age 62 and a paternal grandmother had also died of the disease. The patient had smoked briefly while in college and overall her respiratory status was good. However the preceding summer while in Lake City, Colorado (altitude 8970 feet) she had noted some tiredness with exercise, which prompted referral to a well trained pulmonologist in Dallas. Given her family history of emphysema and a CXR consistent with emphysema, an AAT phenotype and level were drawn which revealed a ZZ phenotype and an AAT level of 19mg/dl (normal >83).

There was no clear family history of liver disease, though one of her sons had been diagnosed with Gilbert's disease. Upon her initial visit pulmonary functions were remarkable for a normal FEV1 (1.89L, 88% predicted) but an FEV1/FVC of 49%, consistent with obstruction. Lung volumes revealed moderate air trapping with a total lung capacity of 134% predicted and residual volume 167% predicted. A high resolution CT showed some evidence of basilar emphysema and mild bronchiectasis. A discussion about AAT replacement therapy ensued.



Alpha-1 Antitrypsin: The Basics

Initial recognition of AAT deficiency was made by Laurel and Eriksson in 1963[2] when they noted that 5/1500 serum protein electrophoreses in Sweden over a 6 month period contained a markedly reduced alpha-1 globulin. Of the 5 individuals demonstrating this pattern, three had developed emphysema at an early age and one had a family history of emphysema. In 1969 Sharp[3] described cirrhosis in 10 children with decreased alpha-1 globulin. While liver disease is more common than lung disease in AAT deficient individuals, the picture of the lung being "digested" by unrestrained protease activity is a powerful image seared into the minds of medical students, physicians and patients alike. Yet the mechanism by which lung disease occurs in this disorder remains obscure nearly 50 years later.



A good story, well told: Carl Bertel Laurel who discovered AAT deficiency and Lloyd Rigler the entrepreneur behind the papain based Adolf's Meat Tenderizer

Similar to other serine protease inhibitors (serpin) AAT is a *B*-sheet protein which is prone to mutations leading to alteration of protein function and polymerization of monomeric structure. The gene coding for AAT is SERPINA 1, a 12.2 kb nucleotide with four coding and three noncoding regions located on chromosome 14 (14q31-32.3). A number of different phenotypes for AAT, including null variants, have been described [4,5](Table 1). The normal MM phenotype (PiM) is found in excess of 80% of the population and is associated with the production of a 394 amino acid molecule yielding serum levels of 80-220 mg/dl, with the majority of protein

produced and excreted by the liver. Although a number of variants of the M allele have been described, with rare exception these are not associated with altered function leading to disease.

The two most common mutations are the S and Z alleles, both of which are characterized by a single amino acid substitution. The S allele contains a valine instead of a glutamic acid at position 264. This protein undergoes rapid degradation within the hepatocyte and is associated with reduced circulating levels of circulating AAT. However the double heterozygote SS phenotype is not associated with specific clinical dysfunction. The Z allele is produced by substitution of lysine for glutamic acid at position 342; the resulting protein has a lower binding constant for its primary target, neutrophil elastase. More importantly the Z protein tends to polymerize intracellularly within the hepatocyte, as well as within the alveolar space (Figure 1). **ZZ homozygotes (PiZ) are the most common individuals to develop clinical disease and will thus be the major focus of this review.** While SZ heterozygotes may have 15-25% normal levels of circulating AAT, the link to clinical disease is less well established.

TABLE 1

Approximate Frequency of AAT Genes and Phenotypes in North America

Gene Frequency			Phenotype					
M	S	Z	PiMM	PiMS	PiMZ	PiSS	PiSZ	PiZZ
95%	3.2%	0.9%	84%	6%	2%	1%	< 1%	<1%

Adapted from[5]

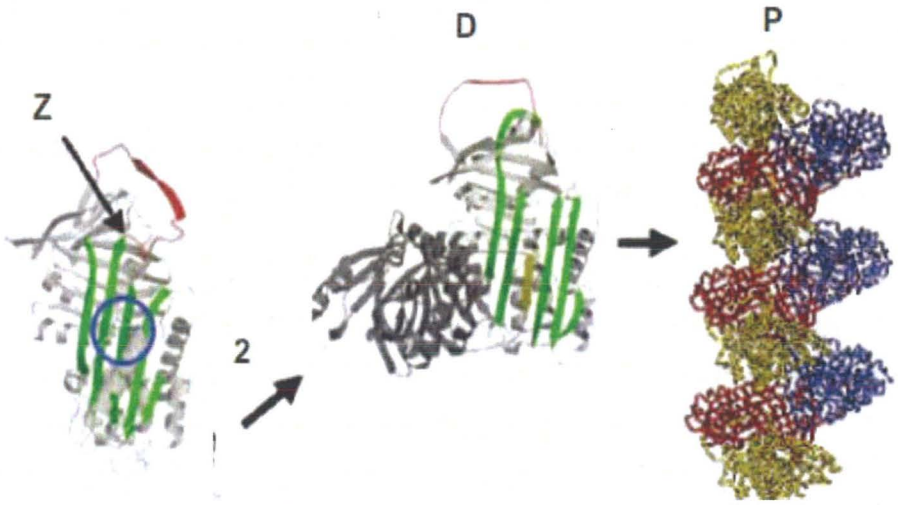


Figure 1 [6] The open *B*-sheet A of the Z protein accepts the loop of another Z molecule to form a dimer (D) and then extends into a polymer (P).

Estimating the incidence of AAT has proved challenging. Using the Hardy-Weinberg equation for estimating deficiency alleles by an indirect epidemiologic approach, roughly 33,000 ZZ individuals exist in the United States, while 173,000 exist worldwide. In excess of 1 million individuals with the SZ phenotype would exist[7]. Population based screening studies of newborns in Oregon yielded 21 ZZ individuals out of 107,000 screened for an incidence of 1 in 5,000[8]. Ethnic differences in allele frequency are well described, with the Z allele prevalence higher in western and northern Europe[9]; indeed the frequency of ZZ in screened Swedish newborns was 1 in 1600[10].

Through a combination of screening and epidemiologic methods the number of ZZ individuals in the United States has been recently estimated at 70,000[11]. It is apparent that the majority of AAT deficient individuals never come to medical attention. Estimates of affected individuals with pulmonary disease actually being diagnosed with AAT deficiency range between 1-4%[9,12], with profound delays in diagnosis being the rule[13].

Production, processing, and modification of AAT

Classically AAT deficiency has been divided into two separate pathways. The first is a toxic "gain of function" where misfolded protein in the liver forms aggregates of polymers injurious to the hepatocyte (Figure 2). The second is the lack of anti-protease activity leading to unrestrained elastolytic activity in lung and other organs. Recently the multiple pathways through which aberrant AAT is processed by the liver have become clearer.

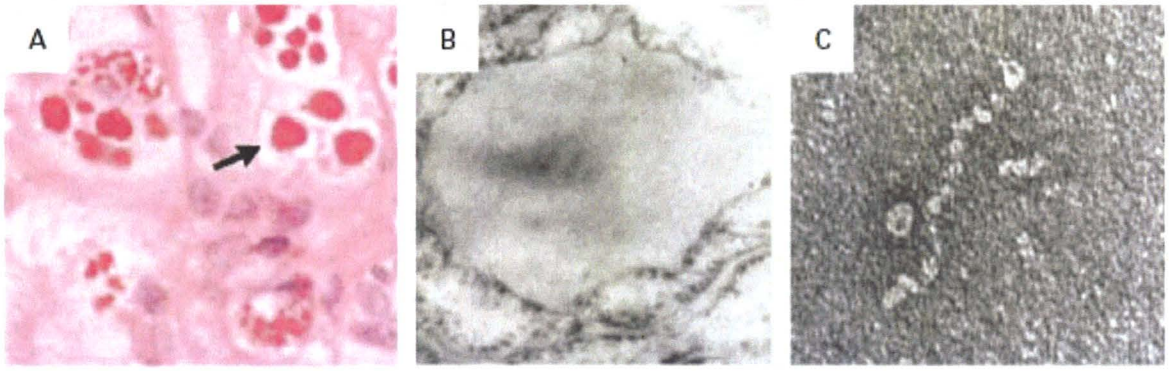


Figure 2[14] Hepatocytes from Z homozygote stained with PAS (Panel A) demonstrate globules containing Z polymers. EM shows a large inclusion in the endoplasmic reticulum(B) and intrahepatic Z polymers giving a bead like structure(C).

Liver

A major function of the endoplasmic reticulum (ER) is to prevent release of misfolded proteins into the secretory pathway. Misfolded glycoproteins are targeted to the proteosome for degradation, a process referred to as ERAD (endoplasmic reticulum associated degradation). The major chaperone required to escort the Z protein out of the endoplasmic reticulum (ER) is calnexin[15]. Prolonged association of calnexin with glucose-containing, misfolded glycoproteins appears integral to targeting to the proteosome for further degradation (figure 3). Other chaperones, such as binding immunoglobulin protein (BiP) belong to the heat shock protein family. When the aberrant protein load exceeds the supply of ER chaperones, protein may aggregate in the ER essentially shutting down the secretory pathways of the cell and producing a condition known as ER stress. Similar mechanisms of disease have been described in a growing number of conditions(including multiple myeloma, neurodegenerative diseases, congenital diabetes, atherosclerosis, and ankylosing spondylitis) characterized by misfolded or unfolded proteins. Protein folding stress may trigger an "unfolded protein response" (UPR) with an extensive signaling pathway trying to balance protein production. The results of this process are extensive and may lead to enhanced autophagy, protein folding, and apoptosis.

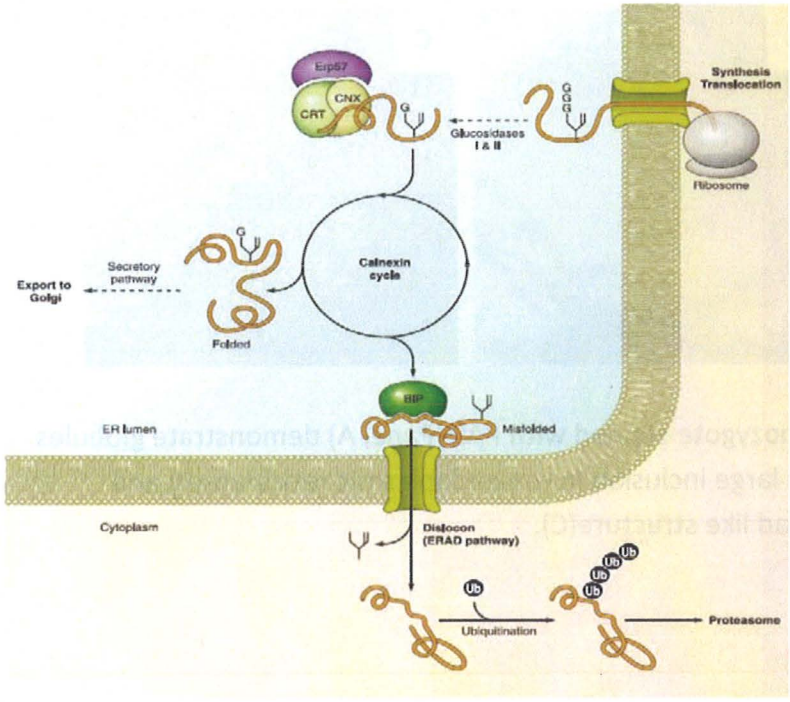


Figure 3[16] Glycosylated proteins in the ER fold in association with the lectin chaperones calnexin(CNX) or calreticulin(CRT). Misfolded proteins are not immediately released from CNX and ultimately bind to BiP(also known as Grp78). Through the ERAD pathway these proteins are dislocated across the ER membrane for ubiquitination and proteosome mediated degradation.

However it is important to note that the relationship of the Z protein to the unfolded protein response (UPR) is complex. While accumulation of Z monomers can induce an UPR, accumulation of Z protein polymers in the ER does NOT activate a classic unfolded protein response[17-19], thought in large part to be due to the highly ordered nature of the polymers. As AAT liver disease is histologically and biochemically characterized by the accumulation of Z polymers, it is apparent that the ER stress produced in this disease is atypical and may actually explain the occurrence of clinical liver disease in only a distinct minority of ZZ patients. The UPR may be initiated in such patients however by a "second hit" such as infection, drugs, or accumulation of other unfolded proteins[20].

In contrast autophagy appears central to the proper disposition of Z protein[21]. Autophagy is a degradative process whereby vesicles which engulf intracellular organelles and cystosol fuse with a lysosome and allow for recycling of intracellular protein. Autophagy appears particularly important for disposing of polymerized and aggregated forms of the Z protein[19]. Recently the administration of carbamazepine (tegretol), an anticonvulsant which has been shown to enhance autophagy, to a transgenic mouse expressing Z protein in the liver resulted in an

increase in intracellular degradation of the Z protein, attenuated hepatic Z protein content, and diminished hepatic fibrosis[22]. An NIH trial utilizing carbamazepine for liver disease in Z homozygotes is currently underway.

Toxic gain of function and lack of function can be produced by the same substance

Lung

AAT is the major defense against neutrophil elastase in the lower respiratory tract. In addition AAT inhibits cathepsin G, proteinase 3, and can complex and inactivate trypsin, chymotrypsin and thrombin. Chronic obstructive pulmonary disease in general, and emphysema in particular, is characterized by an influx of neutrophils and other inflammatory cells into the airways and alveolar space. In AAT deficient individuals with emphysema a marked neutrophilia has been described[23]. As less than 15% of PiZ is actually secreted into the circulation by the liver, and the Z protein has considerably less activity against neutrophil elastase, a "loss of function" leading to an imbalance in proteases and antiproteases with resultant destruction of lung matrix has formed the central hypothesis of emphysema in AAT deficient individuals. Inflammatory pathways produced by cigarette smoke or other agents are amplified by the lack of AAT. Binding of free neutrophil elastase to alveolar macrophages results in release of leukotriene B4 and IL-8 with resultant chemotaxis of neutrophils[24]. The importance of AAT as an anti-inflammatory molecule has gained significant credence as well by observations that sustained expression of AAT can prevent autoimmune disease in mouse models of encephalomyelitis[25]. Additionally AAT interferes with the maturation of dendritic cells and is capable of reducing graft-versus-host disease in models of allogeneic bone marrow

transplantation[26]. Thus reconstitution of AAT levels in the lower respiratory tract should be an attractive strategy to counterbalance inflammation and lung injury leading to emphysema.

However attention has also focused on the possible "toxic gain of function" of Z polymers in the alveolar space, alveolar wall, and interstitium which are readily found in ZZ individuals with emphysema[23,27]. These polymers are inactive as proteinase inhibitors and are strongly chemotactic for neutrophils[28]. The polymerization of Z protein induced by cigarette smoke through oxidative modification of the Z protein has been described in a mouse model of PiZ disease and was tightly correlated with recruitment of neutrophils into the lung[29]. Indeed the observation that Z polymers are present in the interstitium has lead some authors to hypothesize that local production of aberrant protein in the lung contributes to the inflammatory state[30]. Even if this does not occur, since AAT enters the lung through passive diffusion from capillary to alveolar space polymeric Z protein in the interstitium is likely omnipresent, and could explain the panacinar distribution of emphysema.

Disease Associated with AAT Deficiency

Liver

The accumulation of AAT protein with resultant aggregation and polymerization causes clinical liver disease in approximately 10-20% of Z homozygotes[10,31,32]. In a Swedish study of neonates, 18% of PiZ infants had evidence of liver dysfunction, with 10% exhibiting neonatal jaundice. Follow-up of these patients revealed that 25% of those with liver dysfunction died within the first decade of life, and 2% of the survivors developed cirrhosis in childhood. When the surviving patients were surveyed at age 30, 3-5% had abnormalities of liver function but none had clinical liver disease. Another series with an 11 year follow-up of PiZ patients found cirrhosis in 12% and hepatocellular carcinoma in 3%. However an autopsy series of Z homozygotes found histological evidence of cirrhosis in 50% of subjects[33]. Overall AAT deficient PiZ patients accounted for 1.1% of the individuals receiving liver transplants in the United States during the period 1995-2004, with 5 year survival rates of 83%[34].

Transgenic PiZ mice expressing human AAT-Z have been a useful model for studying liver disease from AAT deficiency[22]. Liver disease characterized by AAT containing globules, inflammation, dysplasia, fibrosis, and hepatocellular carcinoma closely mimic disease in humans[35]. The model differs somewhat from human disease in that normal circulating levels of the murine ortholog of AAT are found, though evidence of Z polymers in the lung have been described suggesting that at least some of the human Z protein is secreted systemically[29].

Lung

AAT deficiency is classically associated with early onset emphysema. Those with a smoking history develop more severe disease, at an earlier age than could be expected from smoking alone, with clinical disease presenting in the third or fourth decade of life[36]. Smoking status is integrally linked to rate of decline in lung function amongst Z homozygotes[37] as well as survival. Non-smoking PiZ subjects in Sweden identified by screening and followed for 16 years did not have an increased mortality risk compared to the general population[38]. In contrast PiZ smokers identified either through screening or after clinical presentation with lung disease or non-respiratory disease had a markedly greater mortality risk[39] (figure 4). Thus the importance of smoking avoidance or cessation in this group of patients cannot be overemphasized.

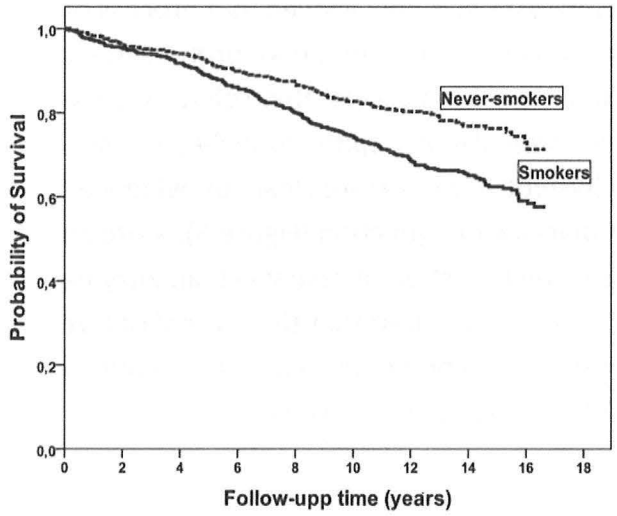


Figure 4 Probability of survival in Z homozygotes regardless of method of diagnosis. Smoking was associated with a markedly worse prognosis[39]

Although early onset emphysema, particularly in a patient with a family history of lung disease, should prompt consideration of AAT deficiency, it is clear that the majority of Z homozygotes with lung disease have a clinical presentation indistinguishable from routine COPD[40]. The characteristic plain radiographic appearance of lower lobe emphysema is seen in only 20% of patients presenting with lung disease[41] and a third of patients have upper lobe predominant emphysema on CT scan[37].

Use of mouse models to study AAT-related emphysema has been hampered by the fact that while humans have only one AAT gene, mice have a family of 3-5 AAT isoforms clustered on murine chromosome 12[42]. Knockout of one specific gene in mice to remove all murine AAT,

with subsequent reconstitution with a human transgene (such as in the PiZ mouse), has not been feasible. Indeed attempts to manufacture a knockout of serpin1a, one of the murine AAT isoforms, leads to embryonic demise[43].

A number of mouse strains differ in susceptibility to experimental smoke-induced emphysema, but a clear relationship to AAT levels has not been established[44,45]. However the production of emphysema in mice with lung specific transgenes has been described in numerous models[46-48]. Indeed it is a well recognized experimental limitation of transgenic mouse models for lung disease that care must be taken to not induce stress in alveolar epithelial cells leading to emphysema[49]. Nevertheless these models have yielded insight into exactly what underlies emphysema in many settings.

Rather than a result of "digested lung matrix" emphysema is now viewed as a process of accelerated aging within the lung[50]. A complex interaction of inflammation, proteases, oxidants, apoptosis, and in some cases telomere dysfunction[51] results in accelerated cell turnover and ultimately a depletion of stem cells capable of renewing alveolar epithelium. Regardless of the system utilized, transgenic mice developing emphysema demonstrate clear cut evidence of ongoing apoptosis in cells residing at the bronchiolar-alveolar junction (Figure 5), a site known to harbor lung stem cells. Integral to this process is the induction of caspase-3, an enzyme which promotes lung epithelial cell apoptosis. It is now recognized that the normal M form of AAT (but not polymeric Z AAT) may play a significant role in preventing caspase-3 induced apoptosis[52,53], suggesting an additional role for AAT in alveolar homeostasis.

Other types of lung disease have also been described in patients with AAT deficiency. Foremost amongst these is bronchiectasis. In one series of PiZ individuals 27% had clinically significant bronchiectasis on CT scan, and a subgroup had this disproportionately to signs of emphysema[54]. In contrast given that AAT phenotypes associated with severe loss of AAT levels are rare, and bronchiectasis is a common disease, it is not surprising that investigation of a heterogeneous group of patients with bronchiectasis revealed no association between bronchiectasis and AAT phenotype[55]. Consideration of AAT deficiency in patients with unexplained bronchiectasis may be useful in some individuals[56].

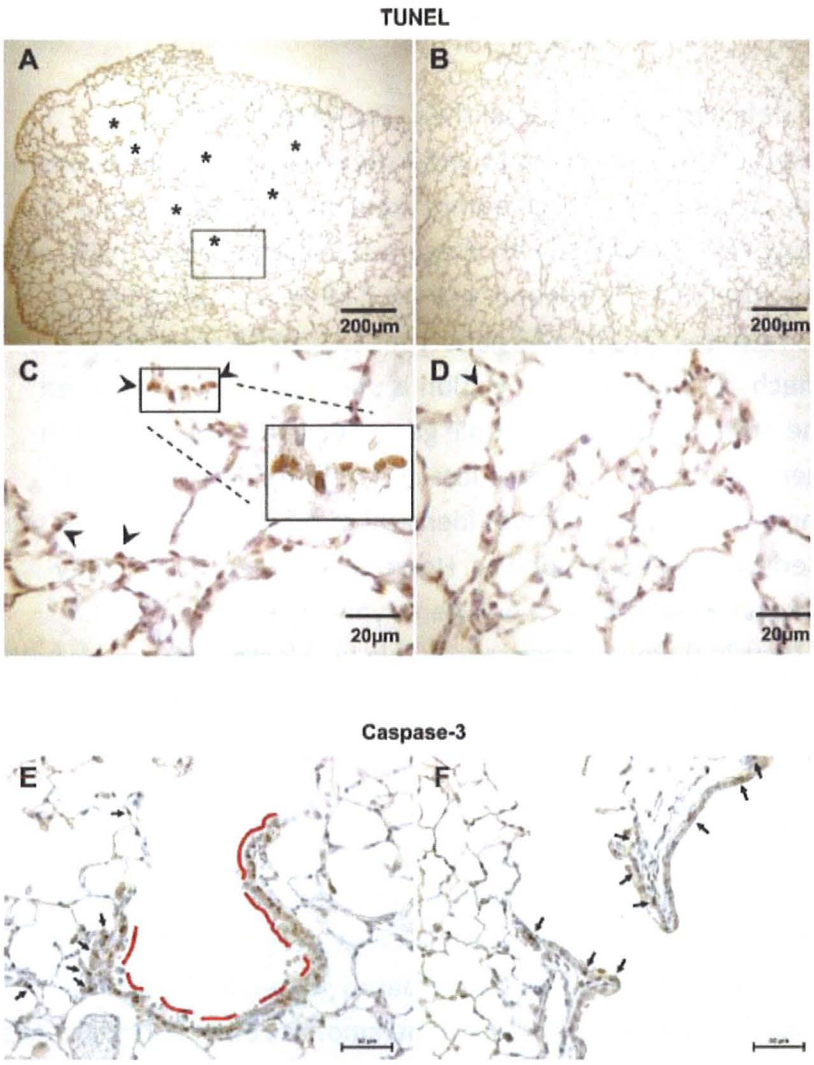


Figure 5 Mice expressing an inducible, lung specific human transgene for PLAGL2, a transcription factor for surfactant protein-C, develop emphysema (panel A), and show signs of ongoing apoptosis in a TUNEL assay (panel C) and caspase-3 up-regulation (panel E) at the bronchoalveolar duct junction and adjacent alveoli. Mice with an uninduced transgene are shown in panels B, D, and F[48].

Skin

There are less than 100 cases of AAT associated panniculitis described in the literature. Areas of fat necrosis in otherwise normal appearing tissue is usually diagnosed on biopsy. While ZZ polymers and neutrophils have been found in these areas[57], it is important to note that panniculitis has been observed in SS and SZ individuals[58,59] and appears to respond well to augmentation with high dose AAT replacement therapy[57]. Taken together these observations suggest that panniculitis is due to a "loss of function" mechanism associated with reduced levels of AAT rather than a "toxic gain of function" from ZZ polymers.

ANCA-associated vasculitis

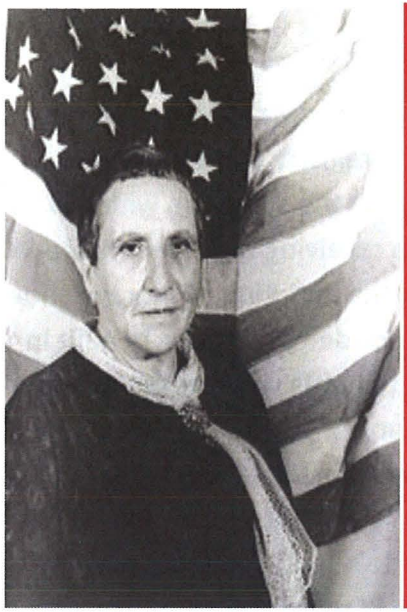
Vasculitis in individuals with AAT deficiency is well established. The vasculitis is usually associated with a c-ANCA, and always due to antibodies to antiproteinase 3 (PR3). Case control studies revealed a 5-18% frequency of the Z allele (both homozygoytes or heterozygotes) in anti-PR3 individuals[60]. A recent study of 433 patients with Wegeners Granulomatosis (WG) and 421 ethnically matched controls disclosed a 7.4% frequency of the Z allele and an 11.5% frequency of the S allele. The **odds ratio** for ZZ, SZ, or SZ genotypes was 14.58 compared to control subjects[61]. Although the mechanism for this association is unclear it should be noted that PR3 is a substrate for AAT. The observation that multiple genotypes are associated with WG would again suggest that either reduced AAT levels or loss of function of the normal M protein (the odds ratio for WG among MM individuals was identical to the control group) is the driving force of the vasculitis caused by anti-PR3 antibodies. However another recent study suggests that only the Z allele is associated with WG [62]. AAT genotyping (via PCR) or phenotyping (using isoelectric gel focusing) would seem reasonable in selected individuals with WG. Use of AAT augmentation therapy in patients with WG and AAT deficiency has not been rigorously explored.

Treatment of AAT Deficiency

The cornerstone of therapy for AAT deficient individuals in general and Z homozygotes in particular is smoking avoidance or cessation[11,56]. Those with clinical evidence of COPD should receive standard therapy for the disease according to evidence based guidelines[63]. Alcohol avoidance and immunization against viral hepatitis should also be emphasized in PiZ individuals.

Many novel therapies for liver disease in PiZ patients are being explored and are geared to reducing the intracellular Z polymer concentration. These include autophagy inducers[22,64], gene therapy with inhibitory RNA [65,66], and chaperone augmentation designed to accelerate transport of the Z protein out of the hepatocyte. Liver transplantation for individuals with significant hepatic dysfunction or hepatocellular carcinoma is now a realistic therapy for many patients.

The major controversy in AAT deficiency continues to revolve around the indications for AAT augmentation. There are now multiple commercial products available for infusion capable of restoring functional AAT to the lower respiratory tract. However the cost is daunting, with estimates in most settings exceeding $100,000/year for the duration of a patients life.



Even if there is a there, there.... do the benefits merit the cost? After all a billion here, a billion there and pretty soon you're talking real money.

Efficacy of AAT Replacement Therapy

1. *Reconstitution of antiprotease levels*

Replacement therapy for AAT was first approved by the FDA in 1987 based on evidence that serum levels of AAT could be raised above a level thought to be protective (80mg/dl) by weekly infusion of 60 mg/kg of AAT[67]. This was associated with increased anti-elastase activity in the lower respiratory tract. There are now 6 commercially available products utilized in the United States for infusion. While replacement therapy is available in Canada and many European nations, AAT replacement is currently not licensed in the UK, Australia, and New Zealand which await further proof of efficacy. Clinical trial design has been difficult, and at times looked upon as a rationale why biochemical demonstration of efficacy of AAT replacement should suffice. An analysis published in 2000 of a potential prospective, randomized study capable of detecting a 40% reduction in mortality at 5 years in individuals with FEV1 between 35-49% predicted, estimated that 684 patients would need to be enrolled over a 2 year period[68]. Given that as of 1998 the NHLBI Registry for Individuals with Severe Deficiency of AAT contained a total of 1,129 patients, 747 of whom were already on therapy[69], the prospects of performing such a large clinical trial seemed remote.

2. *Observational studies*

The impact of AAT replacement therapy has been suggested by some observational studies but the results are open to interpretation. Patients in the NHLBI registry on replacement therapy showed no overall difference in lung function decline, but the subgroup with FEV1 between 30-64% predicted had a slower decline and the group receiving treatment had a lower mortality[69]. A study of 96 patients (85/96 PiZ) before and after the initiation of therapy showed an overall benefit in terms of FEV1 decline but specifically did not demonstrate a benefit in patients with FEV1 between 30-64%[70]. However it did suggest patients with preserved function benefited from augmentation. A more recent observational study of 164 ZZ patients in the Alpha-1 AAT Foundation tissue bank disclosed a significant benefit in those receiving replacement therapy. However, it was noteworthy that of the 40 patients not on therapy the percentage of current smokers was significantly higher (38% v 18%, $p<0.001$) compared to the group receiving augmentation[71]. In both this study and the NHLBI registry study it was noted that individuals with well preserved lung function who received therapy actually did worse.

3. *Randomized trials*

The first randomized clinical trial for AAT replacement occurred in 1999, twelve years after FDA approval. 58 ex-smokers were randomized to every 4 week replacement with AAT or an albumin placebo[72]. No statistically significant improvement in lung function was observed. An observation that there was a trend towards a slower loss of lung tissue on lung CT scan lead to a larger trial, the EXACTILE study (exacerbations and computed tomography scan as lung end-points). 77 PiZ patients were randomized to once weekly augmentation therapy for over 2 years[73]. The primary endpoint was a comparison of lung density by one of 4 methods of CT analysis. A trend towards improvement was seen (p value 0.049-0.084). No difference in lung function or exacerbation frequency was observed. A Cochrane database analysis of the available trials stated that AAT replacement lacked efficacy[74]. This analysis has been criticized over the "pool-ability" of trials using different administration schedules. Similar criticism would seem to be reasonable however for meta-analyses which have pooled all available studies on AAT replacement [75] or have reanalyzed the two randomized trials[76] and found a benefit, leading to the contention that placebo groups in future studies would be unethical. Using a range of estimates of efficacy and a standard definition of cost-effectiveness ($50,000 per quality adjusted life-year) all authors [6,11,77,78] agree that AAT replacement therapy exceeds this threshold and that better therapy is warranted.

In consideration of the above it is difficult to consistently apply the 2003 guidelines for AAT replacement issued by the American Thoracic Society(ATS)[79]. In general these guidelines support replacement for any individual (null, PiZZ, PiSZ) with AAT levels less than 50 mg/dl (11uM) OR any PiZ individual with any evidence of airflow limitation, while realizing that the

data even in observational studies for benefit in patients with FEV1 >50-60% predicted or <35% predicted is unclear. The ATS guidelines also provide extensive guidance for screening populations for AAT deficient alleles. These recommendations suggest phenotyping be performed for all symptomatic adults with COPD, all symptomatic adults with asthma and incompletely reversible airflow obstruction, all asymptomatic individuals with persistent obstruction on PFTs, as well as those with unexplained liver disease or necrotizing panniculitis. Given that there are approximately 15 million patients with COPD in the United States testing in this group alone (retail charge $260/test) would cost in excess of $3.8 billion.

Moreover it is clear that mass screening would likely produce a diagnosis of an "abnormal" AAT phenotype in many patients. The consequences of this were described by the Medical and Scientific Advisory Committee of the Alpha-1 Foundation[80] in an article warning of inappropriate use of AAT replacement. The Alpha-1 Foundation DNA and Tissue Bank at the University of Florida had received 352 samples of material from patients with the MZ phenotype, who don't have an increased risk of lung disease. Of these patients 23 (6.5%) were on replacement therapy. Given that there are approximately 10 million MZ Americans[5], extrapolated to a larger scale AAT replacement would cost roughly $65 billion/year in this population alone if these results were reproduced in a completely screened population. Aggressive promotion of at least one AAT replacement formulation has already drawn the ire of the FDA which specifically objected to claims that replacement therapy "protects the lung" or "slows down any decline in lung function" (http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/ComplianceActivities/Enforcement/UntitledLetters/ucm207335.htm) .

Our Patient

The patient was hesitant to commit to weekly infusion of AAT for her remaining lifespan, even though the ATS Guidelines would have recommended AAT replacement therapy based on her serum AAT levels, CT scan, and evidence of airflow obstruction. Since 2007 she has been treated with standard therapy for COPD and has experienced a total of 3 exacerbations warranting prednisone and antibiotics. Her activities are unlimited.

PFTS:	2007	2012
FEV1	1.89(88%pred)	1.78(86%)
DLCO	15.35(92%)	13.58(79%)

Bibliography

1. Kubba AK, Young M. The Long Suffering of Frederic Chopin. Chest 1998;113:210-6.
2. Laurell CB, Eriksson S. The electrophoretic alpha-1 globulin pattern of serum in alpha-1 antitrypsin deficiency. Scand J clin Lab Invest 1963;15:132-40.
3. Sharp HL BR, Krivit W, Freier EF, Krivit. Cirrhosis associated with alpha-1 antitrypsin deficiency: a previously unrecognized inherited disorder. Scand J Clin lab Invest 1969;73:934-9.
4. DeMeo DL, Silverman EK. α1-Antitrypsin deficiency · 2: Genetic aspects of α1-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk. Thorax 2004;59:259-64.
5. de Serres FJ. Worldwide Racial and Ethnic Distribution of α1-Antitrypsin Deficiency. Chest 2002;122:1818-29.
6. Dickens JA LD. Why has it been so difficult to prove the efficacy of alpha-1-antitrypsin replacemnt therapy? Insights from the study of disease pathogenesis. Drug Design, Development and Therapy 2011;5:391-405.
7. De Serres FJ BI, Fernandez-Bustillo E. Estimates of PiS and PiZ alpha-1 antitrypsin deficiency alleles prevalence in the carribean, north, central, and south america. Monaldi Arch Chest Dis 2009;71:96-105.
8. O'Brien ML, Buist NRM, Murphey WH. Neonatal screening for alpha1-antitrypsin deficiency. The Journal of Pediatrics 1978;92:1006-10.
9. Luisetti M, Seersholm N. α1-Antitrypsin deficiency · 1: Epidemiology of α1-antitrypsin deficiency. Thorax 2004;59:164-9.
10. Sveger T. Liver Disease in Alpha1-Antitrypsin Deficiency Detected by Screening of 200,000 Infants. New England Journal of Medicine 1976;294:1316-21.
11. Stoller JK, Aboussouan LS. A Review of α1-Antitrypsin Deficiency. American Journal of Respiratory and Critical Care Medicine 2012;185:246-59.
12. Silverman EK, Miletich JP, Pierce JA, et al. Alpha-1-Antitrypsin Deficiency: High Prevalence in the St. Louis Area Determined by Direct Population Screening. American Journal of Respiratory and Critical Care Medicine 1989;140:961-6.
13. Stoller JK, Sandhaus RA, Turino G, Dickson R, Rodgers K, Strange C. Delay in Diagnosis of α1-Antitrypsin Deficiency. Chest 2005;128:1989-94.
14. Carrell RW, Lomas DA. Alpha1-Antitrypsin Deficiency — A Model for Conformational Diseases. New England Journal of Medicine 2002;346:45-53.
15. Cameron PH, Chevet E, Pluquet O, Thomas DY, Bergeron JJM. Calnexin Phosphorylation Attenuates the Release of Partially Misfolded α1-Antitrypsin to the Secretory Pathway. Journal of Biological Chemistry 2009;284:34570-9.
16. Hetz C MF, Rodriguez D, and L. H. Glimcher. The unfolded protein response: Integrating stress signals through the stresssensor IRE1alpha. Physiol Rev 2011;91:1219-43.
17. Hidvegi T, Schmidt BZ, Hale P, Perlmutter DH. Accumulation of Mutant α1-Antitrypsin Z in the Endoplasmic Reticulum Activates Caspases-4 and -12, NFκB, and BAP31 but Not the Unfolded Protein Response. Journal of Biological Chemistry 2005;280:39002-15.
18. Hidvegi T, Mirnics K, Hale P, Ewing M, Beckett C, Perlmutter DH. Regulator of G Signaling 16 Is a Marker for the Distinct Endoplasmic Reticulum Stress State Associated with Aggregated Mutant α1-Antitrypsin Z in the Classical Form of α1-Antitrypsin Deficiency. Journal of Biological Chemistry 2007;282:27769-80.

19. Kamimoto T, Shoji S, Hidvegi T, et al. Intracellular Inclusions Containing Mutant α1-Antitrypsin Z Are Propagated in the Absence of Autophagic Activity. Journal of Biological Chemistry 2006;281:4467-76.
20. Davies MJ Me, Roussel BD, Kaufman RJ, Marciniak SJ, Lomas DA. Neuroserpin polymers activate NF-kappaB by a calcium signalling pathway that is independent of the unfolded protein response. J Biol Chem 2009;284:18202-9.
21. Perlmutter DH. The Role of Autophagy in Alpha-1-Antitrypsin Deficiency: A Specific Cellular Response in Genetic Diseases Associated with Aggregation-Prone Proteins. Autophagy 2006;2:258-63.
22. Hidvegi T, Ewing M, Hale P, et al. An Autophagy-Enhancing Drug Promotes Degradation of Mutant α1-Antitrypsin Z and Reduces Hepatic Fibrosis. Science 2010;329:229-32.
23. Mahadeva R, Atkinson C, Li Z, et al. Polymers of Z α1-Antitrypsin Co-Localize with Neutrophils in Emphysematous Alveoli and Are Chemotactic in Vivo. The American Journal of Pathology 2005;166:377-86.
24. Woolhouse IS, Bayley DL, Stockley RA. Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of α1–antitrypsin deficiency and the role of leukotriene B4 and interleukin 8. Thorax 2002;57:709-14.
25. Subramanian S, Shahaf G, Ozeri E, et al. Sustained expression of circulating human alpha-1 antitrypsin reduces inflammation, increases CD4+FoxP3+ Treg cell population and prevents signs of experimental autoimmune encephalomyelitis in mice. Metabolic Brain Disease 2011;26:107-13.
26. Tawara I, Sun Y, Lewis EC, et al. Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proceedings of the National Academy of Sciences 2012;109:564-9.
27. Elliott PR, Bilton D, Lomas DA. Lung Polymers in Z α1-Antitrypsin Deficiency-related Emphysema. Am J Respir Cell Mol Biol 1998;18:670-4.
28. Parmar JS, Mahadeva R, Reed BJ, et al. Polymers of α1-Antitrypsin Are Chemotactic for Human Neutrophils. Am J Respir Cell Mol Biol 2002;26:723-30.
29. Alam S, Li ZJ, Janciauskiene S, Mahadeva R. Oxidation of Z alpha(1)-Antitrypsin by Cigarette Smoke Induces Polymerization A Novel Mechanism of Early-Onset Emphysema. Am J Respir Cell Mol Biol 2011;45:261-9.
30. Gooptu B, Ekeowa UI, Lomas DA. Mechanisms of emphysema in α1-antitrypsin deficiency: molecular and cellular insights. European Respiratory Journal 2009;34:475-88.
31. Hussain M, Mieli-Vergani G, Mowat AP. α1-antitrypsin deficiency and liver disease: Clinical presentation, diagnosis and treatment. Journal of Inherited Metabolic Disease 1991;14:497-511.
32. Bernspång E, Carlson J, Piitulainen E. The liver in 30-year-old individuals with alpha1-antitrypsin deficiency. Scandinavian Journal of Gastroenterology 2009;44:1349-55.
33. Eriksson S, Carlson J, Velez R. Risk of Cirrhosis and Primary Liver Cancer in Alpha1-Antitrypsin Deficiency. New England Journal of Medicine 1986;314:736-9.
34. Kemmer N, Kaiser T, Zacharias V, Neff GW. Alpha-1-Antitrypsin Deficiency: Outcomes After Liver Transplantation. Transplantation Proceedings 2008;40:1492-4.
35. Marcus NY, Brunt EM, Blomenkamp K, et al. Characteristics of hepatocellular carcinoma in a murine model of alpha-1-antitrypsin deficiency. Hepatology Research 2010;40:641-53.
36. McElvaney NG, Brantly ML, Stoller JK, et al. Baseline Characteristics of Enrollees in the National Heart, Lung and Blood Institute Registry of α1-Antitrypsin Deficiency. Chest 1997;111:394-403.
37. Parr DG, Stoel BC, Stolk J, Stockley RA. Pattern of Emphysema Distribution in α1-Antitrypsin Deficiency Influences Lung Function Impairment. American Journal of Respiratory and Critical Care Medicine 2004;170:1172-8.
38. Tanash HA, Nilsson PM, Nilsson J-Å, Piitulainen E. Clinical course and prognosis of never-smokers with severe alpha-1-antitrypsin deficiency (PiZZ). Thorax 2008;63:1091-5.

39. Tanash H, Nilsson P, Nilsson J-A, Piitulainen E. Survival in severe alpha-1-antitrypsin deficiency (PiZZ). Respiratory Research 2010;11:44.
40. Tobin MJ, Cook PJL, Hutchison DCS. Alpha1antitrypsin deficiency: The clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z: A survey by the British Thoracic Association. British Journal of Diseases of the Chest 1983;77:14-27.
41. Gishen P, Saunders AJS, Tobin MJ, Hutchison DCS. Alpha1-antitrypsin deficiency: the radiological features of pulmonary emphysema in subjects of Pi type Z and Pi type SZ: a survey by the British thoracic association. Clinical Radiology 1982;33:371-7.
42. McNab GL, Dafforn TR, Wood A, Sapey E, Stockley RA. A novel model and molecular therapy for Z alpha-1 antitrypsin deficiency. Mamm Genome 2012;23:241-9.
43. Wang DM, Wang WM, Dawkins P, et al. Deletion of serpina1a, a murine alpha(1)-antitrypsin ortholog, results in embryonic lethality. Exp Lung Res 2011;37:291-300.
44. CAVARRA E, BARTALESI B, LUCATTELLI M, et al. Effects of Cigarette Smoke in Mice with Different Levels of α1-Proteinase Inhibitor and Sensitivity to Oxidants. American Journal of Respiratory and Critical Care Medicine 2001;164:886-90.
45. Guerassimov A, Hoshino Y, Takubo Y, et al. The Development of Emphysema in Cigarette Smoke-exposed Mice Is Strain Dependent. American Journal of Respiratory and Critical Care Medicine 2004;170:974-80.
46. Baron RM, Choi AJS, Owen CA, Choi AMK. Genetically manipulated mouse models of lung disease: potential and pitfalls. American Journal of Physiology - Lung Cellular and Molecular Physiology 2012;302:L485-L97.
47. Shapiro SD. The use of transgenic mice for modeling airways disease. Pulm Pharmacol Ther 2008;21:699-701.
48. Yang YS, Yang MCW, Guo YH, Williams OW, Weissler JC. PLAGL2 expression-induced lung epithelium damages at bronchiolar alveolar duct junction in emphysema: bNip3-and SP-C-associated cell death/injury activity. Am J Physiol-Lung Cell Mol Physiol 2009;297:L455-L66.
49. Perl AK, Zhang LQ, Whitsett JA. Conditional Expression of Genes in the Respiratory Epithelium in Transgenic Mice Cautionary Notes and Toward Building a Better Mouse Trap. Am J Respir Cell Mol Biol 2009;40:1-3.
50. Tuder RM, Yoshida T, Arap W, Pasqualini R, Petrache I. State of the Art. Cellular and Molecular Mechanisms of Alveolar Destruction in Emphysema. Proceedings of the American Thoracic Society 2006;3:503-10.
51. Amsellem V, Gary-Bobo G, Marcos E, et al. Telomere Dysfunction Causes Sustained Inflammation in Chronic Obstructive Pulmonary Disease. American Journal of Respiratory and Critical Care Medicine 2011;184:1358-66.
52. Petrache I, Fijalkowska I, Medler TR, et al. α-1 Antitrypsin Inhibits Caspase-3 Activity, Preventing Lung Endothelial Cell Apoptosis. The American Journal of Pathology 2006;169:1155-66.
53. Petrache I, Fijalkowska I, Zhen L, et al. A Novel Antiapoptotic Role for α1-Antitrypsin in the Prevention of Pulmonary Emphysema. American Journal of Respiratory and Critical Care Medicine 2006;173:1222-8.
54. Parr DG, Guest PG, Reynolds JH, Dowson LJ, Stockley RA. Prevalence and Impact of Bronchiectasis in α1-Antitrypsin Deficiency. American Journal of Respiratory and Critical Care Medicine 2007;176:1215-21.
55. Cuvelier A, Muir J-F, Hellot M-F, et al. Distribution of α1-Antitrypsin Alleles in Patients With Bronchiectasis. Chest 2000;117:415-9.
56. American Thoracic Society/European Respiratory Society Statement. American Journal of Respiratory and Critical Care Medicine 2003;168:818-900.

57. Gross B, Grebe M, Wencker M, Stoller JK, Bjursten LM, Janciauskiene S. New Findings in PiZZ α1-Antitrypsin Deficiency-Related Panniculitis. Dermatology 2009;218:370-5.
58. Chng WJ, Henderson CA. Suppurative panniculitis associated with alpha 1-antitrypsin deficiency (PiSZ phenotype) treated with doxycycline. British Journal of Dermatology 2001;144:1282-3.
59. Pinto AR, Maciel LS, Carneiro F, Resende C, Chaves FC, Freitas AF. Systemic nodular panniculitis in a patient with alpha-1 antitrypsin deficiency (PiSS phenotype). Clinical and Experimental Dermatology 1993;18:154-5.
60. Esnault VLM, Testa A, Audrain M, et al. Alpha1-antitrypsin genetic polymorphism in ANCA-positive systemic vasculitis. Kidney Int 1993;43:1329-32.
61. Mahr AD EJ, Stone JH, Hoffman GS, et al. Alpha-antitrypsin deficiency-related alleles Z and S and the risk of Wegener's granulomatosis. Arthritis Rheum 2010;62:3760-7.
62. Morris H, Morgan MD, Wood AM, et al. ANCA-associated vasculitis is linked to carriage of the Z allele of α1 antitrypsin and its polymers. Annals of the Rheumatic Diseases 2011;70:1851-6.
63. Qaseem A, Wilt TJ, Weinberger SE, et al. Diagnosis and Management of Stable Chronic Obstructive Pulmonary Disease: A Clinical Practice Guideline Update from the American College of Physicians, American College of Chest Physicians, American Thoracic Society, and European Respiratory Society. Annals of Internal Medicine 2011;155:179-91.
64. Hidvegi T, Mukherjee A, Ewing M, Kemp C, Perlmutter DH. Chapter Three - The Role of Autophagy in Alpha-1-Antitrypsin Deficiency. In: James CW, Phillip IB, eds. Methods in Enzymology: Academic Press; 2011:33-54.
65. Cruz PE, Mueller C, Cossette TL, et al. In vivo post-transcriptional gene silencing of [alpha]-1 antitrypsin by adeno-associated virus vectors expressing siRNA. Lab Invest 2007;87:893-902.
66. Li CW, Xiao PJ, Gray SJ, Weinberg MS, Samulski RJ. Combination therapy utilizing shRNA knockdown and an optimized resistant transgene for rescue of diseases caused by misfolded proteins. Proc Natl Acad Sci U S A 2011;108:14258-63.
67. Wewers MD, Casolaro MA, Sellers SE, et al. Replacement Therapy for Alpha1-Antitrypsin Deficiency Associated with Emphysema. New England Journal of Medicine 1987;316:1055-62.
68. SCHLUCHTER MD, STOLLER JK, BARKER AF, et al. Feasibility of a Clinical Trial of Augmentation Therapy for α1-Antitrypsin Deficiency. American Journal of Respiratory and Critical Care Medicine 2000;161:796-801.
69. The AAT deficiency study group. Survival and FEV1 Decline in Individuals with Severe Deficiency of α1-Antitrypsin. American Journal of Respiratory and Critical Care Medicine 1998;158:49-59.
70. Wencker M, Fuhrmann B, Banik N, Konietzko N. Longitudinal Follow-up of Patients With α1-Protease Inhibitor Deficiency Before and During Therapy With IV α1-Protease Inhibitor. Chest 2001;119:737-44.
71. Tonelli A, Rouhani F, Li N, Schreck P, Brantly M. Alpha 1 antitrypsin augmentation therapy in deficient individuals enrolled in the Alpha-1 Foundation DNA and Tissue Bank. Int J Chron Obstruct Pulmon Dis 2009;4:443-52.
72. DIRKSEN A, DIJKMAN JH, MADSEN F, et al. A Randomized Clinical Trial of α1-Antitrypsin Augmentation Therapy. American Journal of Respiratory and Critical Care Medicine 1999;160:1468-72.
73. Dirksen A, Piitulainen E, Parr DG, et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in α1-antitrypsin deficiency. European Respiratory Journal 2009;33:1345-53.
74. Gotzsche PC JH. Intravenous alpha-1 antitrypsin augmentation therapy for treating patients with alpha-1 antitrypsin and lung disease. Cochrane Database Syst Rev 2010;CD007851.
75. Chapman KR, Stockley RA, Dawkins C, Wilkes MM, Navickis RJ. Augmentation Therapy for α1 Antitrypsin Deficiency: A Meta-Analysis. COPD: Journal of Chronic Obstructive Pulmonary Disease 2009;6:177-84.

76. Stockley RA pD, Piitulainen E, Stolk J, Stoel B, Dirksen A. Therapeutic efficacy of alpha-1 antitrypsin augmentation therapy on the loss of lung tissue: an integrated analysis of 2 randomized clinical trials using computed tomography densitometry. Respiratory Research 2010;11:136-44.
77. Alkins SA, O'Malley P. Should Health-Care Systems Pay for Replacement Therapy in Patients With α1-Antitrypsin Deficiency?. Chest 2000;117:875-80.
78. Gildea TR, Shermock KM, Singer ME, Stoller JK. Cost-Effectiveness Analysis of Augmentation Therapy for Severe α1-Antitrypsin Deficiency. American Journal of Respiratory and Critical Care Medicine 2003;167:1387-92.
79. American Thoracic Society/European Respiratory Society Statement: Standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. Am J Respir Crit Care Med 2003;168:818 - 900.
80. Sandhaus RA, Turino G, Stocks J, et al. α1-Antitrypsin Augmentation Therapy for PI*MZ Heterozygotes. Chest 2008;134:831-4.